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Health and Environmental Effects Profile for Direct Black 38

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR DIRECT BLACK 38

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16. ABSTRACT

The Health and Environmental Effects Profile for Direct Black 38 was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Direct Black 38 has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for Direct Black 38 is 8.6 $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for Direct Black 38 is 100.

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1985.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Reference doses (RfDs) for chronic exposures. An RfD is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, RfDs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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EXECUTIVE SUMMARY

Direct Black 38 is the common name for a commercial azo dye, which is produced by the coupling of benzidine with 8-amino-1-naphthol-3,6-disulfonic acid under acidic conditions, coupling aniline to this complex under alkaline conditions, coupling the intermediate with m-phenylenediamine, and neutralizing with NaOH. It is soluble in water, moderately soluble in ethanol and ethylene glycol monoethyl ether, and insoluble in most other organic solvents (Society of Dyers and Colourists, 1971). It is used commercially to dye textiles, leather and paper (IARC, 1982a). Five U.S. manufacturers produced 3.759 million pounds in 1976 (USITC, 1977), but only one U.S. manufacturer reported production of Direct Black 38 in 1981 (USITC, 1982a). Six U.S. companies currently market Direct Black 38 under various tradenames (AATCC, 1985). In 1983, ~148,000 pounds of this dye was imported into the United States through principal customs districts (USITC, 1984).

Very little experimental data specific to Direct Black 38 are available to predict its environmental fate. Two BOD studies (Stafford and Northup, 1955; Atlantic Chemical Co., 1982) using Direct Black 38 indicated relatively poor biodegradation. Yoshida et al. (1981) observed degradation of Direct Black 38 in aqueous solution with either natural river water or soil inocula; however, no degradation was observed using sterilized river water or soil, which suggested occurrence of microbial transformation. If released to water, Direct Black 38 may be susceptible to significant adsorption because dyestuffs by their substantative nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). Removal of adsorbed Direct Black 38 may also

occur by anaerobic biodegradation since a number of azo dyes have been found to be substantially biodegraded under anaerobic conditions (Brown and Laboureur, 1983). Direct dyes are relatively stable to direct photolysis in natural waters (Porter, 1973); however, humic materials in natural water were found to strongly accelerate the photodecomposition of azo dyes (Haag and Mill, 1985). Reduction of the dye to aniline and benzidine derivates may occur in water containing H_2S, SO_2 or other reducing agents (Takemura et al., 1965). Hydrolysis, volatilization and bioconcentration are not expected to be significant. Therefore, indirect photolysis, microbial degradation and adsorption are the important fate processes in water. If released to the atmosphere, Direct Black 38 will probably be associated with particulate matter and dusts that are subject to wet and dry deposition. It may be oxidized by OH radical present in the atmosphere. If released to soil, Direct Black 38 may not leach significantly, since dyestuffs are susceptible to significant adsorption. Transformation in soil may occur microbially, and photodegradation beyond the surface layer of soil may not be important.

Occupational exposure to Direct Black 38 has and probably still occurs during its production and use in dyeing (IARC, 1982a). Wastewater effluents are probably the major source of release to the environment. Ambient monitoring data could not be located in the available literature; however, occupational levels in the range of 1.6-5.1 mg/m³ have been recorded in a paper-dyeing facility (IARC, 1982a).

Little et al. (1974) reported that the 96-hour LC_{50} of Direct Black 38 to fathead minnows (Pimephales promelas) was >180 mg/ℓ. This greatly exceeds the concentrations used to produce color (0.4-1.5 mg/ℓ). No other information regarding aquatic toxicity of Direct Black 38 was found.

Studies concerning the amount or rate of gastrointestinal absorption of Direct Black 38 were not available. Rabbits absorbed at least 91% of the radioactivity from a dermally applied dose of ^{14}C-Direct Black 38 (IBM, 1979).

Pertinent data concerning the distribution of Direct Black 38 could not be located in the available literature as cited in the Appendix.

Direct Black 38 is metabolized to benzidine and acetylated benzidines. In vitro experiments with S-9 and isolated rat hepatocytes indicated that the production of benzidine is catalyzed by microsomal enzymes, while the subsequent acetylation of benzidine is catalyzed by cytosolic enzymes (Bos et al., 1984). Benzidine and acetylated benzidines have been detected in the urine of workers exposed to benzidine-based dyes (NIOSH, 1980) and rats, mice, monkeys and hamsters orally exposed to Direct Black 38 (NCI, 1978; Rinde and Troll, 1975; Nony and Bowman, 1980).

Direct Black 38 was demonstrated to be carcinogenic in mice and rats by the oral route. Mice exposed to 0.3% Direct Black 38 in their drinking water for 55-60 weeks had increased incidences of hepatomas and mammary carcinomas compared with controls (Asada et al., 1981). NCI (1978) observed a statistically significant increased liver tumor incidence for male and female rats given 1500 ppm of Direct Black 38 in the diet for 91 days. No liver tumors were observed at lower doses or in controls. Time to first tumor was 4 and 13 weeks in male and female rats, respectively, as compared with ~78 weeks in historic controls. No liver tumors were observed in mice; however, foci of cellular alteration of the liver were observed in three mice at 12,500 ppm. The duration of treatment may not have been long enough for mice to develop tumors.

IARC (1982a) reported several Japanese drinking water studies for Direct Black 38. Negative results were obtained in rats given 100 mg/ℓ for 60 weeks (Okajima et al., 1975) and 400 mg/ℓ for 14 months (Niitsu 1973). Two papillomas and three carcinomas of the urinary bladder, three carcinomas of the liver and two adenocarcinomas of the colon were obtained in 6/13 rats given 500 mg/ℓ for 60 weeks (Okajima et al., 1975). Negative results were obtained in a pulmonary adenoma bioassay in mice or when Direct Black 38 (in wax pellets) was implanted into the bladder of mice (Maronpot et al., 1983; Niitsu, 1973). Yoshida and Miyakawa (1973) and Yoshida et al. (1971) conducted a case control study of 200 male cases of bladder cancer and 148 male controls of the same age with urinary disorders. Seventeen of the bladder cancer cases and two of the controls worked in the silk-dyeing industry, which had a relative risk of 6.8 ($p=0.002$). Seven of the 17 bladder cancer cases were silk painters who maintained oral contact with their work tools. Genin (1977) found bladder tumors in workers occupationally exposed to direct azo dyes and detected benzidine in the urine of exposed workers. IARC (1982a,b) concluded that exposures to benzidine-based dyes were strongly associated with the occurrence of bladder cancer in humans. Although the epidemiological data were inadequate to evaluate the possible carcinogenic effect to man of Direct Black 38 alone, when considered together with data demonstrating that benzidine was present in the urine of occupationally exposed workers, it provided "sufficient evidence that occupational exposure to benzidine-based dyes represents a carcinogenic risk to man."

Direct Black 38 was mutagenic in the Salmonella typhimurium reverse mutation assays (Prival et al., 1984; Joachim et al., 1985; Reid et al., 1984), and the presence of S-9 and FMN or bacterial reduction enhanced the mutagenic activity of the compound.

No data concerning the teratogenic effects of Direct Black 38 after oral or inhalation exposure was available. Wilson (1955) subcutaneously injected pregnant rats with 30 mg of Direct Black 38 and observed that 19/91 implantations were resorbed and no malformations occurred. Control data was not provided. Pertinent data regarding the reproductive effects of Direct Black 38 could not be located in the available literature as cited in the Appendix.

The subchronic toxicity of Direct Black 38 has been studied by NCI (1978) where rats and mice were exposed to concentrations of 190-3000 and 750-12,500 ppm, respectively. Among the effects observed in rats of both sexes were cirrhotic liver alterations, which included biliary hyperplasia at $\geq$750 ppm and nodular regeneration and portal fibrosis at 1500 ppm. Other toxic effects observed in rats were lymphoid depletion of the spleen and myeloid depletion of the bone marrow at $\geq$1500 ppm (both sexes), lymphoid depletion of the thymus in males at levels $\geq$1500 ppm and 3000 ppm in females, and subacute glomerulonephritis at 1500 ppm (both sexes). None of these lesions were found in controls. At 3000 ppm, all rats of both sexes died and six males at 1500 ppm died before the end of the study. Several toxic effects of Direct Black 38 were observed in mice. At concentrations $\geq$6000 ppm, mice of both sexes exhibited pigment deposits in the liver, hemosiderosis of the kidney and pigment deposits in the thyroid. At a concentration of 1500 ppm in mice of both sexes, hemosiderosis of the spleen was observed. Hepatocellular degeneration and biliary hyperplasia was observed in mice of both sexes at 3000 and 12,500 ppm, respectively.

A q_1^* of 8.6 $(mg/kg/day)^{-1}$ for Direct Black 38 was derived based on the incidence of liver tumors in female rats maintained on diets containing up to 1500 ppm Direct Black 38 for 91 days in the NCI (1978) study.

Concentrations in water corresponding to increased lifetime risk levels of 10^{-5}, 10^{-6} and 10^{-7} are $4x10^{-5}$, $4x10^{-6}$ and $4x10^{-7}$ mg/ℓ, assuming a 70 kg human consumes 2 ℓ water/day. An F factor of 86 $(mg/kg/day)^{-1}$ was also derived from the female rat tumor data in the NCI study. The F factor places Direct Black 28 in Potency Group 2. Evidence that Direct Black 38 alone is carcinogenic in humans is inadequate but there is sufficient evidence that it is carcinogenic in animals. However, a strong association of occupational exposure to benzidine-based dyes and human bladder cancer, together with the presence of benzidine in the urine of exposed individuals, is supportive of an EPA Group A ranking (i.e., human carcinogen) for benzidine-based dyes of which Direct Black 38 is one. A Group A chemical in Potency Group 2 has a HIGH hazard ranking under CERCLA. An RQ of 100 was derived from the data for increased mortality in male rats treated with 1500 ppm Direct Black 38 in the diet for 91 days in the NCI (1978) study.

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LIST OF ABBREVIATIONS

BOD	Biological oxygen demand
BODT	Biological oxygen demand, theoretical
bw	Body weight
CAS	Chemical Abstract Service
COD	Chemical oxygen demand
DMSO	Dimethyl sulfoxide
EC-GC	Electron capture-gas chromatography
FMN	Flavin mononucleotide
HPLC	High pressure liquid chromatography
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients (and all other subscripted dose levels)
LD_{50}	Median lethal dose
MED	Minimum effective dose
MTD	Maximum tolerated dose
ppb	Parts per billion
ppm	Parts per million
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value

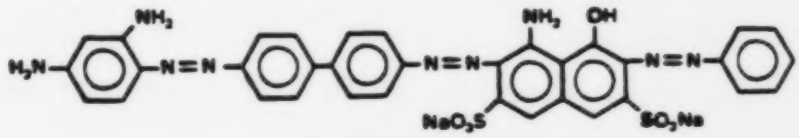
1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Direct Black 38 is a common name for the compound disodium 4-amino-3-{[4'-((2,4-diaminophenyl)azo)-4-biophenyl]azo}-5-hydroxy-6-(phenylazo)-2,7-naphthalene disulfonate, according to the IUPAC System. The current CAS designation for Direct Black 38 is 2,7-naphthalenedisulfonic acid, 4-amino-3-{[4'-((2,4-diaminophenyl)azo)-(1,1'-biphenyl)-4-yl]azo}-5-hydroxy-6-(phenylazo)-, disodium salt. Direct Black 38 is currently marketed in the United States under the following tradenames (AATCC, 1985):

Amidine Black GANB 200% (John Campbell & Co.)
Chemdirect Black GX (Chem-Mark, Inc.)
Direct Black E 200% (Fabricolor, Inc.)
Direct Black GAC Conc. 200% (Blackman Uhler Chem.)
Direct Black GX 200% (Fabricolor, Inc.)
Direct Black SD 200% (American Cyanamid)
Elcomine Black GX-NB (International Dyestuffs Corp.)
Elcomine Direct Black GXOO 200% (International Dyestuffs Corp.)

The structure, molecular weight, empirical formula and CAS number are as follows:



Molecular weight: 781.7

Empirical formula: $C_{34}H_{25}N_9Na_2O_7S_2$

CAS number: 1937-37-7

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Direct Black 38 is a grey-black powder (IARC, 1982a). It is soluble in water, moderately soluble in ethanol and ethylene glycol monoethyl ether and insoluble in most other organic solvents (Society of Dyers and Colorists, 1971). All direct dyes are water soluble and form anions by dissociation

(Kuehni et al., 1979). In aqueous solution, the molecules of direct dyestuffs are linked by hydrogen bonds forming larger agglomerates or colloidal solutions; this reduces their solubility and promotes disposition on cellulosic fibers. The structure of these agglomerates allows firm linkage to the cellulose molecule by multiple hydrogen bonds (Kuehni et al., 1979).

Physical properties such as melting point, boiling point, vapor pressure, density and log K_{ow} could not be located in the available literature. Calculation of log K_{ow} by computer or other methods was not possible because of the lack of appropriate data (U.S. EPA, 1986a). The air conversion factor for Direct Black 38 is 1 ppm = 32.5 mg/m³.

The manufacture and testing of Direct Black 38 do not conform to rigid chemical specifications, and its composition may vary in order to meet shade and intensity requirements (IARC, 1982a). The residual benzidine content of six U.S.-produced samples of Direct Black 38 ranged from 2-20 mg/kg (IARC, 1982a).

1.3. PRODUCTION DATA

Direct Black 38 was first produced in commercial quantities in the United States in 1914 (IARC, 1982a). It is produced by coupling benzidine to 8-amino-1-naphthol-3,6-disulfonic acid under acidic conditions, coupling aniline to this complex under alkaline conditions, coupling the intermediate with m-phenylenediamine, and neutralizing with NaOH (Society of Dyers and Colourists, 1971).

In 1976, five U.S. companies produced 3.759 million pounds of Direct Black 38 (USITC, 1977); this is the most recent U.S. production figure available. In 1981, the last year USITC reported individual production of Direct Black 38, Fabricolor Inc. was listed as the only manufacturer of this dye (USITC, 1982a). Current U.S. companies that market Direct Black 38 are

listed in Section 1.1. The U.S. production data for 1977 available from the public portion of the U.S. EPA TSCA Production File are listed in Table 1-1.

Import of Direct Black 38 through principal U.S. customs districts in recent years has been reported as follows (USITC, 1981, 1982b, 1983, 1984).

Year	Import Volume (pounds)
1980	208,618
1981	221,715
1982	217,557
1983	147,795

1.4. USE DATA

Direct Black 38 is used to dye cellulose, wool, silk, bast, hog's hair, leather, plastics, vegetable-ivory buttons and wood flour used as a resin filler· stain wool, silk, acetate, nylon, wood and biological materials; print cellulose, wool and silk; and produce aqueous inks (IARC, 1982a). It also has been used in hair dyes (Feinland et al., 1980).

Nonbenzidine dye substitutes for Direct Black 38 have been developed for use on cellulosic fibers; however, Direct Black 38 was still being used commercially in 1980 to dye textiles, leather and paper (IARC, 1982a).

1.5. SUMMARY

Direct Black 38 is the common name for a commercial azo dye that is produced by the diazotization of benzidine with 8-amino-1-naphthol-3,6-disulfonic acid under acidic conditions, coupling aniline to this complex under alkaline conditions, coupling the intermediate with m-phenylenediamine, and neutralizing with NaOH. It is soluble in water, moderately soluble in ethanol and ethylene glycol monoethyl ether, and insoluble in most other organic solvents (Society of Dyers and Colourists, 1971). It is used commercially to dye textiles, leather and paper (IARC, 1982a). Five U.S. manufacturers produced 3.759 million pounds in 1976 (USITC, 1977), but

TABLE 1-1

Direct Black 38 Production Data for 1977*

Production, Location	Manufacturer or Importer	Production Range (pounds)
Fabricolor, Paterson, NJ	manufacturer	0.1-1.0 million
GAF Corp., Rensselaer, NY	manufacturer	1-10 million
Ugine Kuhlmann of America, Paramus, NJ	importer	confidential
L & R Dyestuffs, Clifton, NJ	importer	0.1-1.0 million
American Cyanamid, Bound Brook, NJ	importer	1-10 thousand
Atlantic Chemical, Nutley, NJ	importer	confidential

*Source: U.S. EPA, 1977

only one U.S. manufacturer reported production of Direct Black 38 in 1981 (USITC, 1982a). Six U.S. companies currently market Direct Black 38 under various tradenames (AATCC, 1985). In 1983, ~148,000 pounds of this dye was imported into the United States through principal customs districts (USITC, 1984).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Experimental hydrolysis data could not be located in the available literature as cited in the Appendix; however, Direct Black 38 does not contain functional groups readily susceptible to environmental hydrolysis. Therefore, hydrolysis is not expected to be environmentally relevant.

2.1.2. Oxidation/Reduction. Specific experimental data could not be located in the available literature as cited in the Appendix. Takemura et al. (1965) reported that bubbling H_2S through a pure azo-dye solution yields aromatic amines and suggested that azo dyes in wastewater may be reduced at the azo linkage (by H_2S or SO_2 in the water) to form compounds, such as benzidine or naphthylamine, from which the dye was produced. A water system receiving dyes, however, would have to be very polluted so that H_2S or SO_2 (or a reducing environment) would significantly convert the dye. Sediments in water, however, may be naturally anaerobic and provide appropriate reducing conditions.

2.1.3. Photolysis. Porter (1973) examined the aqueous photodegradation rate of 8 direct dyes and 12 other azo dyes in artificial light and one basic dye in natural sunlight. Based on the experimental results, the author concluded that direct dyes are relatively stable to direct photolysis in natural waters. Although Direct Black 38 was not one of the dyes studied, its structure is similar to several dyes that were tested.

Haag and Mill (1985) examined the aqueous photodegradation rate of 15 azo dyes by simulated sunlight or natural sunlight and found that humic materials in natural water strongly accelerate the indirect photodecomposition rate of all the dyes. Therefore, indirect photolysis may be an important removal mechanism for azo dyes in water.

2.1.4. Microbial Degradation. Stafford and Northrup (1955) reported a 5-day BODT of 8% for Direct Black 38 with sewage seed as microbial inoculum. A COD and 5- and 20-day BODs of 965,800, 3600 and 32,400 mg/ℓ, respectively, were reported for Direct Black 38 (Atlantic Chemical Co., 1982), indicating relatively poor biodegradation.

Yoshida et al. (1981) examined the degradation of a dilute aqueous solution of Direct Black 38 in the presence of nutrient broth and incubation with Escherichia coli, river water, sterilized river water, soil or sterilized soil. Within 48 hours, a significant color change was observed in the solutions incubated with Escherichia coli, river water and soil, and benzidine was tentatively identified in the solutions. No color change or benzidine formation was observed in the solutions incubated with sterilized river water or soil. The authors concluded that the Direct Black 38 was microbially reduced at the azo bonds forming benzidine.

Although Direct Black 38 was not one of the dyes tested, Brown and Laboureur (1983) reported that a number of azo dyes were substantially biodegraded under anaerobic test conditions and suggested that the breakdown of dyestuffs in the environment may be initiated under anaerobic conditions.

2.1.5. Volatilization. Experimental data could not be located in the available literature as cited in the Appendix. Direct Black 38 is soluble in water (Society of Dyers and Colourists 1971) and is expected to have a relatively low vapor pressure, based on its chemical structure, which would predict a relatively small Henry's Law constant. Therefore, volatilization from water is not expected to be significant in the environment.

2.1.6. Adsorption. Experimental data specific to Direct Black 38 could not be located in the available literature as cited in the Appendix.

Dyestuffs by their substantive nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). In conventional biological waste treatment systems, soluble dye removal usually occurs when the dye is adsorbed onto sludge (Porter, 1973). Sewage works sludge is usually treated by anaerobic digestion or landfilling. Removal of the dye from the sludge must therefore occur by anaerobic biodegradation. Similar anaerobic conditions exist in many lakes and rivers with respect to adsorbed materials on silts and sediments and, therefore, adsorbed dyes may be subject to anaerobic biodegradation in the environment (Brown and Laboureur, 1983).

2.1.7. Bioconcentration. Experimental data could not be located in the available literature as cited in the Appendix. Since Direct Black 38 is water soluble (Society of Dyers and Colourists, 1971), bioconcentration in aquatic organisms is not expected to be significant.

2.2. AIR

Direct Black 38 in the atmosphere is most likely to be associated with particulate matter and dusts, especially from atmospheric effluents resulting from production and use. These particulate matter and dusts are subject to wet and dry deposition. Aromatic amines react fast (half-life of a few hours) with OH radical in the atmosphere (Atkinson, 1985). Since Direct Black 38 contains aromatic amine groups, it may also undergo oxidation with atmospheric OH radical.

2.3. SOIL

Pertinent experimental data could not be located in the available literature as cited in the Appendix. Chemical degradation in soil may not be significant since dyes, in general, are resistant to oxidative degradation (Porter, 1973) and hydrolysis is not expected to be environmentally

relevant. Yoshida et al. (1981) observed degradation of Direct Black 38 in an aqueous medium using a natural soil inoculum; lack of degradation in the same medium using sterilized soil was indicative of microbial transformation in aquatic media. Anaerobic biodegradation may also be possible (see Section 2.1.4.). Therefore, transformation of Direct Black 38 within soil will most likely occur microbially; however, the rate of biotransformation is not clear. Photodegradation beyond the surface soil layer does not appear to be significant.

Since Direct Black 38 is water soluble (Society of Dyers and Colourists, 1971), leaching in soil might be expected. The substantive nature of dyestuffs (Brown and Laboureur, 1983), however, including the direct dyes (Kuehni et al., 1979), indicates that significant adsorption to soil may occur. Therefore, significant leaching in soil may not occur.

2.4. SUMMARY

Very little experimental data specific to Direct Black 38 are available to predict its environmental fate. Two BOD studies (Stafford and Northup, 1955; Atlantic Chemical Co., 1982) using Direct Black 38 indicated relatively poor biodegradation. Yoshida et al. (1981) observed degradation of Direct Black 38 in aqueous solution with either natural river water or soil inocula; however, no degradation was observed using sterilized river water or soil, which suggested occurrence of microbial transformation. If released to water, Direct Black 38 may be susceptible to significant adsorption because dyestuffs by their substantative nature (ability to be exhaustively deposited to fibers from aqueous baths) are likely to be adsorbed onto both sewage works sludge and silts and sediments of rivers and lakes (Brown and Laboureur, 1983). Removal of adsorbed Direct Black 38 may also occur by anaerobic biodegradation since a number of azo dyes have been found

to be substantially biodegraded under anaerobic conditions (Brown and Laboureur, 1983). Direct dyes are relatively stable to direct photolysis in natural waters (Porter, 1973); however, humic materials in natural water were found to strongly accelerate the photodecomposition of azo dyes (Haag and Mill, 1985). Reduction of the dye to aniline and benzidine derivates may occur in water containing H_2S, SO_2 or other reducing agents (Takemura et al., 1965). Hydrolysis, volatilization and bioconcentration are not expected to be significant. Therefore, indirect photolysis, microbial degradation and adsorption are the important fate processes in water. If released to the atmosphere, Direct Black 38 will probably be associated with particulate matter and dusts that are subject to wet and dry deposition. It may be oxidized by OH radical present in the atmosphere. If released to soil Direct Black 38 may not leach significantly, since dyestuffs are susceptible to significant adsorption. Transformation in soil may occur microbially, and photodegradation beyond the surface layer of soil may not be important.

3. EXPOSURE

Direct Black 38 has not been reported to occur as such in nature (IARC, 1982a); therefore, exposure from natural sources is not expected to occur. Occupational exposure to Direct Black 38 has and probably still does occur during its production and its use for dyeing textiles, leather and paper (IARC, 1982a). Likely routes of exposure are inhalation of particulates and dermal exposure. A survey conducted by NIOSH estimated that ~13,000 workers are exposed to Direct Black 38 (IARC, 1982a).

3.1. WATER

Monitoring data could not be located in the available literature as cited in the Appendix. For the organic dye industry, in general, estimates are that ~90% of the dye ends up on the fabric, while 10% is lost to wastewater effluents during dye operations (Porter, 1973; Brown et al., 1981). Loss of dyes to wastewater effluents during manufacture was estimated to be 1-2% (Brown et al., 1981). Waste streams from dye manufacture contain dissolved inorganic salts and small amounts of dye and dye intermediates (Steadman et al., 1977). Wastewater effluents are therefore the major source of release to natural waters; the efficiency of wastewater treatment operations will determine the amount of dye that reaches natural waters.

Release of dye from textile fabrics may occur from commercial or consumer washings. Dyes in most finished products, however, are considered to be essentially "fast" (they do not migrate or wash out) (Jones, 1979). Various after-treatments are commonly applied to fabrics dyed with direct dyes to improve their colorfastness (Kuehni et al., 1979).

3.2. FOOD

Pertinent food monitoring data could not be located in the available literature as cited in the Appendix.

3.3. INHALATION

Direct Black 38 has been detected in the workplace air of a paper-dyeing facility at a total airborne particulate level of 1.6-5.1 mg/m³ and at an unspecified level at a textile-dyeing operation (IARC, 1982a). Ambient air monitoring data could not be located in the available literature as cited in the Appendix.

Ambient atmospheric emissions may occur from ventilation of effluents at production and use sites. In addition, air effluents from production operations such as spray-drying may be sources of release. Particlate emissions from these sources are usually filtered at the plant site, which should significantly reduce the amount of particulate matter actually reaching the ambient atmosphere.

3.4. DERMAL

Pertinent dermal monitoring data could not be located in the available literature as cited in the Appendix. The general public is exposed mainly to finished dyes after they have been applied to the product; however, there is little chance of dyes coming off in perspiration, saliva, or washings if label instructions are followed (Jones, 1979).

3.5. SUMMARY

Occupational exposure to Direct Black 38 has and probably still occurs during its production and use in dyeing (IARC, 1982a). Wastewater effluents are probably the major source of release to the environment. Ambient monitoring data could not be located in the available literature; however, occupational levels in the range of 1.6-5.1 mg/m³ have been recorded in a paper-dyeing facility (IARC, 1982a).

4. PHARMACOKINETICS

4.1. ABSORPTION

Pertinent data concerning the extent and rate of absorption of Direct Black 38 by the oral and inhalation routes could not be located in the available literature as cited in the Appendix. Absorption by the gastrointestinal tract is indicated, however, since metabolites (Section 4.3.) appear in the urine after oral exposure. In a study by IBM (1979), rabbits were treated dermally with ^{14}C-Direct Black 38 (labeled in the biphenyl moiety) and 91% of the applied radioactivity was recovered in the urine over 144 hours, suggesting that benzidine-based dye was substantially absorbed from the intact skin of the rabbits.

4.2. DISTRIBUTION

Pertinent data concerning the distribution of Direct Black 38 could not be located in the available literature as cited in the Appendix.

4.3. METABOLISM

Studies on the metabolism of Direct Black 38 have been focused mainly on the production of benzidine and benzidine derivatives because of the known carcinogenicity of benzidine (U.S. EPA, 1986d). Metabolism of Direct Black 38 to other products or by other pathways has not been documented.

Urine samples from workers occupationally exposed to benzidine-based dyes including Direct Black 38, were found to contain monoacetyl benzidine, benzidine and diaminobenzidine (NIOSH, 1980; Lowry et al., 1980). Walker (1970) suggested that benzidine-based dyes may be degraded in the gut and liver to free benzidine or monoacetylbenzidine.

In a 13-week toxicity study, NCI (1978) found benzidine and monoacetylbenzidine in urine collected over a 24-hour period from male and female B6C3F1 mice and Fischer 344 rats. The mice had been fed up to 12,000 ppm Direct Black 38, while rats received up to 3000 ppm in their diets.

Rhesus monkeys, fed two different doses of Direct Black 38, equivalent to 13 and 63 mg of benzidine, by stomach tube excreted 0.16 and 0.31 mg, respectively, of benzidine plus monacetylbenzidine in the urine (Rinde and Troll, 1975). The quantities represented 1.3 and 0.5% of the benzidine moiety in Direct Black 38. Since the investigators determined that the Direct Black 38 administered was free of benzidine as an impurity, the benzidine and monoacetylbenzidine found in the urine could only have resulted from the metabolism of Direct Black 38.

Nony and Bowman (1980) studied the metabolism of Direct Black 38 in male Syrian golden hamsters. Fifteen hamsters (3 per metabolism cage) received a single dose of 100 mg/kg Direct Black 38 by oral intubation; three control hamsters were given distilled water. Urine was collected up to 7 days and analyzed for metabolites by EC-GC and HPLC. The identified metabolites were benzidine, monoacetylbenzidine, diacetylbenzidine, 4-aminobiphenyl and alkaline hydrolyzable conjugates of benzidine.

Bos et al. (1984) found that incubation of Direct Black 38 with rat liver 9000 g supernatant resulted in the production of benzidine, but no mono- or diacetylbenzidine. When Direct Black 38 was incubated with rat hepatocytes, however, diacetylbenzidine, but not benzidine or monoacetylbenzidine, was detected. Inocubation of benzidine with hepatocytes resulted in considerable amounts of diacetylbenzidine, but inoculation of benzidine with 9000 g supernatant resulted in the appearance of neglible amounts of monoacetylbenzidine and no diacetylbenzidine. If the 9000 g supernatant was supplemented with acetyl CoA, however, incubation with benzidine produced both mono- and diacetylbenzidine. The results indicate that Direct Black 38 is reduced by an azoreductase system in the microsomes to free benzidine, which is subsequently acetylated in the nonmicrosomal or cytosolic fraction.

4.4. EXCRETION

Data regarding the amount and rate of excretion of Direct Black 38 could not be located in the available literature as cited in the Appendix, but the metabolites, benzidine and acetylated benzidines, are excreted in the urine of occupationally exposed humans (NIOSH, 1980) and orally exposed rats, mice, monkeys and hamsters (NCI, 1978; Rinde and Troll, 1975; Nony and Bowman, 1980).

4.5. SUMMARY

Studies concerning the amount or rate of gastrointestinal absorption of Direct Black 38 were not available. Rabbits absorbed at least 91% of the radioactivity from a dermally applied dose of ^{14}C-Direct Black 38 (IBM, 1979).

Pertinent data concerning the distribution of Direct Black 38 could not be located in the available literature as cited in the Appendix.

Direct Black 38 is metabolized to benzidine and acetylated benzidines. In vitro experiments with S-9 and isolated rat hepatocytes indicated that the production of benzidine is catalyzed by microsomal enzymes, while the subsequent acetylation of benzidine is catalyzed by cytosolic enzymes (Bos et al., 1984). Benzidine and acetylated benzidines have been detected in the urine of workers exposed to benzidine-based dyes (NIOSH, 1980) and rats, mice, monkeys and hamsters orally exposed to Direct Black 38 (NCI, 1979; Rinde and Troll, 1975; Nony and Bowman, 1980).

5. EFFECTS

5.1. CARCINOGENICITY

5.1.1. Animal Studies. Asada et al. (1981) gave a group of 60 (sex not specified), 4-week-old ICR mice weighing 25-30 g Direct Black 38 (purity unspecified) at a concentration of 0.3% in their drinking water for 55-60 weeks. A group of 20 control mice were given water without Direct Black 38 for 60 weeks. Macroscopic examination and histological examination of unspecified organs were performed. The treated mice developed hepatomas and mammary carcinomas (Table 5-1) compared with no tumors of either type in controls; 9/60 mice developed both types of tumors. An additional group of 40 mice were given the same concentration of Direct Black 38 in the drinking water, and starting at 16 weeks, two mice were killed every other week to determine the time course of tumor development. The first liver tumor was observed at week 20; and the first mammary tumor was observed simultaneously with hepatic tumors at week 38.

NCI (1978) gave groups of 10 Fischer 344 rats and 10 B6C3F1 mice of each sex Direct Black 38 (~86% pure with no detectable benzidine contamination; detection limit 0.004% by HPLC) in the diet for 91 days with 2 additional observation days. Rats were exposed to concentrations of 0, 190, 375, 1500 or 3000 ppm (1 ppm benzidine). Mice were treated at 0, 750, 1500, 3000, 6000 (2 ppm benzidine) or 12,500 (4 ppm benzidine) ppm. A dose-related increase in mortality was observed in rats, but not in mice. All rats of both sexes given 3000 ppm of Direct Black 38 and six males given 1500 ppm died before the end of the study. No deaths occurred in the other groups of rats or in the mice. All animals that died, were killed when moribund or killed at the end of the study were necropsied. Microscopic examination of skin, lungs, bone marrow, spleen, mandibular and mesenteric lymph nodes,

TABLE 5-1

Tumor Incidences in ICR Mice Given Direct Black 38
of Unspecified Purity in the Drinking Water for 55-60 Weeks*

Concentration (%)	Target Organ	Tumor Type	Tumor Incidence (%)
0	liver	hepatoma	0/20 (0)
0.3	liver	hepatoma	46/59 (78)
0	mammary	carcinomas	0/20 (0)
0.3	mammary	carcinomas	20/59 (34)

QUALITY OF EVIDENCE

Strengths of Study: Mice were given Direct Black 38 by a relevant route of administration (oral) for an extended period of time (55-60 weeks).

Weaknesses of Study: Whether or not both sexes were tested was not clear and only one concentration was tested. The nature and extent of gross and histopathological examination was not reported. Whether or not the MTD was estimated by adequate pre-testing was not reported.

*Source: Asada et al., 1981

thymus, heart, salivary gland, bile duct (mice), liver, pancreas, stomach, small intestine, colon, kidney, bladder, adrenals, thyroids, testes and epididymis was performed only on controls, rats treated at 1500 and 3000 ppm and mice treated at 12,500 ppm, unless precluded by cannibalism or autolysis. Microscopic examination of other groups of rats was limited to the liver, spleen, bone marrow, kidney, pancreas and salivary gland of 750 ppm rats and the livers of 190 and 375 ppm rats. Microscopic examination of the other groups of mice was limited to the spleen of all groups, the livers of mice at $\geq$1500 ppm and the kidneys and thyroids of mice at $\geq$3000 ppm. Liver tumors were observed in male rats ($p<0.001$) and female rats ($p=0.016$) at 1500 ppm (Table 5-2). Liver tumors were not observed in controls. The time to observation of first tumor was 4 and 13 weeks in male and female rats, respectively, as compared with ~78 weeks in historic controls. The absence of liver tumors at 3000 ppm may have been attributable to the early mortality of these rats. Animals were sacrificed at 13 weeks. Preneoplastic lesions (basophilic foci) occurred in treated rats, but not in controls. Basophilic foci were observed in 3/9 male rats, which also had tumors, and 1/8 female rats given 1500 and 3000 ppm Direct Black 38, respectively. When incidences of foci of cellular alteration (a possible preneoplastic lesion) were added to the incidence of basophilic foci, significant differences in additional dose groups were obtained. The incidences of basophilic foci or foci of cellular alteration in males were 7/10 at 375 ppm ($p=0.002$), 9/10 at 750 ppm ($p<0.001$) and 5/9 at 1500 ppm ($p=0.011$) and in females were 5/9 at 375 ppm ($p=0.011$), 10/10 at 750 ppm ($p<0.001$), 10/10 at 1500 ppm ($p<0.001$) and 1/8 at 3000 ppm. None of these lesions were observed at 190 ppm or in controls. No increased incidences of liver tumors or other tumors occurred in mice; however, foci of cellular alteration of the liver were observed in

TABLE 5-2

Incidence of Liver Tumors in Fischer 344 Rats Given 190-3000 ppm of Direct Black 38 (no benzidine contamination) in the Diet for 91 Days and Observed for an Additional 2 Days[a]

Concentration (ppm)	Sex	Tumor Incidence[b] (p value)[c]
0	M	0/10
190	M	0/10
375	M	0/10
750	M	0/10
1500	M	9/9 (p<0.001)
3000	M	0/9
0	F	0/10
190	F	0/10
375	F	0/10
750	F	0/10
1500	F	5/10 (p=0.016)
3000	F	0/8

QUALITY OF EVIDENCE

Strengths of Study: Both sexes of two animal species (rats and mice) were tested by a relevant route of administration (dietary) at several concentrations. The MTD was estimated by pre-testing and data were statistically analyzed. Histopathological examination was comprehensive at higher doses and limited at lower doses.

Weakness of Study: The experiment did not last for the lifespan of the animals tested.

Overall Adequacy: Sufficient

[a]Source: NCI, 1978

[b]In males 4/9 liver tumors were hepatocellular carcinomas and 5/9 were neoplastic nodules. In females, all five tumors were neoplastic nodules.

[c]Fisher Exact test

three mice at 12,500 ppm. Treatment may not have been long enough for mice to develop tumors, a conclusion supported by the Asada et al. (1981) study, in which induction of tumors in mice occurred after 55-60 weeks of oral exposure.

Two Japanese drinking water studies were reviewed by IARC (1982a). Okajima et al. (1975) gave a group of 12 Wistar rats 100 mg/ℓ commercial Direct Black 38 (benzidine-free as determined by HPLC) in their drinking water and observed no tumors in the 8 rats that were still alive at 60 weeks. These investigators also gave a group of 15 rats 500 mg/ℓ Direct Black 38 in drinking water. Two papillomas and three carcinomas of the urinary bladder, three carcinomas of the liver and two adenocarcinomas of the colon were observed in 6/13 treated rats surviving until week 60. Also, hyperplasia of the bladder mucosa and liver were observed in nine and eight of the survivors, respectively. No tumors were observed in nine control rats. Niitsu (1973) gave a group of 20 male and 25 females rats 400 mg/ℓ Direct Black 38 (purity not specified) in drinking water for 14 months; only 4 males and 2 females survived the treatment period. One female had breast cancer; no other neoplasm was reported. The number of control animals was not specified.

A few studies by less relevant routes of administration were available as well. Direct Black 38 gave negative results in the mouse Strain A pulmonary adenoma bioassay by the intraperitoneal route of administration (Maronpot et al., 1983). A high frequency of false negatives (78%) were obtained for the aromatic amines in this test system. Niitsu (1973) implanted wax pellets containing 10% Direct Black 38 into the bladders of 50 female dd mice and observed bladder carcinoma in 1/21 treated mice alive after 40 weeks. An incidence of one bladder carcinoma in 36 control mice was observed.

5.1.2. Human Studies. No studies of Direct Black 38 alone could be located in the available literature as cited in the Appendix. IARC (1982a) summarized numerous case studies reporting the occurrence of bladder tumors in dyestuff workers, as well as several epidemiological studies suggesting excess mortality from bladder cancer in workers occupationally exposed to benzidine-based dyes. Interpretation of these studies are difficult because the subjects were exposed to mixtures of several dyes. IARC (1982a) reviewed and evaluated a Japanese hospital-based case control study of 200 males with bladder cancer and 148 male controls of the same age with urinary disorders (Yoshida and Myakawa, 1973; Yoshida et al., 1971). Seventeen (8.5%) males with bladder cancer and two (1.4%) of the controls worked in the silk-dyeing industry; the relative risk for employment in the silk-dyeing industry was 6.8 (p=0.002). Seven of the seventeen bladder cancer cases were silk painters who maintained oral contact with their work tools, thus ingesting the dyes. One of the dyes commonly used by the painters was Direct Black 38.

Genin (1977) detected benzidine (trace amounts to 0.3 µg/mL) in the urine of 22 workers occupationally exposed to direct azo dyes. The specific dyes were not reported, although Direct Black 38 and Direct Blue 2 were referred to as being the most important dyes commercially. A retrospective analysis of plant records found five cases of bladder cancer in an unspecified number of workers. Three of the five cases had been exposed for 3-24 years with a latency period of 18-43 years. Levels of exposure were not reported. IARC (1982a,b) concluded that exposure to benzidine-based dyes was strongly associated with the occurrence of bladder cancer in humans. Although the epidemiological data were inadequate to evaluate the possible carcinogenic effect of Direct Black 38 alone, when considered together with

data demonstrating that benzidine was present in the urine of occupationally exposed workers, the data provided "sufficient evidence that occupational exposure to a mixture of benzidine-based dyes represent a carcinogenic risk to man" (IARC, 1982a,b).

5.2. MUTAGENICITY

Direct Black 38 has been tested for reverse mutation activity in Salmonella typhimurium TA98 and TA1538 (Table 5-3). Direct Black 38 was mutagenic in assays by Prival et al. (1984), Joachim et al. (1985) and Reid et al. (1984) in the presence of rat or hamster liver S-9. Reduction of the azo bond using dithionite (Joachim et al., 1985) or flavin mononucleotide (Prival et al., 1984; Reid et al., 1984) resulted in a slight increase in mutagenic activity, while reduction by overnight incubation with rat cecal bacteria greatly increased the mutagenic activity (Reid et al., 1984). Joachim et al. (1985) also showed that a urine concentrate from rats intubated with a single dose of 500 mg/kg was mutagenic with rat S-9 in both TA1538 and TA98.

5.3. TERATOGENICITY

No data were available in the literature as cited in the Appendix regarding teratogenic effects of oral or inhalation exposure to Direct Black 38.

Wilson (1955) studied the teratogenic effects of several azo dyes including Direct Black 38. Laparotomies were perfomed on 16 Wistar rats on gestation day 7 to confirm pregnancy and count implantation sites; the rats were injected subcutaneously with 1 mL of a 1% aqueous dye solution on gestation days 7, 8 and 9. The total dose of dye received by each rat (weighing 200-250 g) was 30 mg (120-150 mg/kg). Control group data were not provided. Dams were killed on gestation day 20, and the number of resorption sites and live fetuses were counted. Fetuses were placed in Bouin's

TABLE 5-3

Reverse Mutation Mutagenicity Testing of Direct Black 38

Indicator/ Organism	Compound and/or Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Salmonella typhimurium TA98	dissolved in DMSO	plate incorporation	0.1-0.3 µmol/plate	+S-9 + FMN +S-9 - FMN	+ +	FMN reduction, increased mutagenic activity; S-9 from Aroclor-induced hamster liver	Prival et al., 1984
S. typhimurium TA1538, TA98	dissolved in DMSO or distilled water	plate incorporation	NR	+S-9 + dithionite +S-9 - dithionite	+	S-9 from Aroclor-induced rats; dithionite reduction resulted in little increase.	Joachim et al., 1985
S. typhimurium TA1538	in ethylacetate and DMSO	plate incorporation	0.25-1.0 µmol/plate	+S-9 rat ± bacterial reduction +S-9 hamster + FMN	+ + +	Bacterial reduction enhanced mutagenic activity	Reid et al., 1984
S. typhimurium TA1538, TA98	urine extract from rats	plate incorporation	0.5-2.5 mL urine/plate	±S-9	±	Positive dose response with S-9	Joachim et al., 1985

NR = Not reported

fluid or 95% alcohol for later examination. Of the pregnancies that continued to the 20th day, 19/91 implantations were resorbed; no malformations were found in the remaining 72 fetuses. Three dams died before the 20th day of gestation.

5.4. OTHER REPRODUCTIVE EFFECTS

Pertinent data regarding the reproduction effects of Direct Black 38 could not be located in the available literature as cited in the Appendix.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

NCI (1978) gave groups of 10 Fischer 344 rats and 10 B6C3F1 mice of each sex dietary Direct Black 38 for 91 days and for 2 additional observation days. The rats were exposed to concentrations of 190, 375, 750, 1500 and 3000 ppm; the mice were exposed to concentrations of 750, 1500, 3000, 6000 and 12,500 ppm (see Section 5.1.). Rats of both sexes had a dose-related decreased mean body weight gain at $\geq$750 ppm. All male and female rats at 3000 ppm and six male rats at 1500 ppm died. No deaths occurred in the other group of rats. The authors reported several trends in the data for liver lesions for rats, which revealed a progression of change from a normal to a preneoplastic condition to neoplasia for the dyes in general. The rats surviving for longer periods of time had more proliferative liver changes as well as preneoplastic and neoplastic lesions than the rats that were moribund at earlier times. Livers that were more severely affected were of two types: a hyperplastic liver with multiple foci of cellular alteration and nodules, and a cirrhotic liver with nodules. The first type of liver changes was considered preneoplastic and neoplastic (see Section 5.1.). Cirrhosis (nodular regeneration and biliary hyperplasia, focal) and biliary hyperplasia occurring along with neoplastic nodules and carcinomas can be considered nonneoplastic lesions. Biliary hyperplasia was observed in males

and females at 750 ppm. Nodular regeneration and portal fibrosis were observed in rats of both sexes at 1500 ppm. These cirrhotic changes occurred in the longer-lived rats. Nonneoplastic lesions with more rapid onset included lymphoid depletion of the spleen and thymus, myeloid depletion of the bone marrow and varying degrees of biliary hyperplasia. Lymphoid depletion of the spleen and myeloid depletion of the bone marrow occurred in rats of both sexes at ≥1500 ppm. Lymphoid depletion of the thymus was observed in males at ≥1500 ppm and females at 3000 ppm. At 1500 ppm, subacute glomerulonephritis was observed in rats of both sexes. None of these lesions were found in controls. Several toxic effects of Direct Black 38 were observed in mice. High-dose male and female mice had slightly reduced body weight gains compared with controls. All mice survived until the end of the study. At concentrations ≥6000 ppm, 100% of the mice of both sexes exhibited pigment deposits in the liver and hemosiderosis of the kidney and 8/10 males and 10/10 females had pigment deposits in the thyroid. Hemosiderosis of the spleen was observed in 100% of the mice at ≥3000 ppm; in 4/10 males and 9/10 females at 1500 ppm and 1/10 males and 1/10 females at 750 ppm. At concentrations ≥3000 ppm, hepatocellular degeneration was observed in 90-100% of the mice of both sexes. Biliary hyperplasia was observed in 9/10 males and 10/10 females at 12,500 in both sexes. None of these lesions were found in controls.

5.6. OTHER RELEVANT INFORMATION

In 2-week toxicity tests to determine the MTD for the 13-week studies, groups of five male and five female B6C3F1 mice and five male and five female Fischer 344 rats were fed Direct Black 38 at dietary levels of 0, 3000, 6000, 12,500, 25,000 or 50,000 ppm (NCI, 1978). Dose-related decreased food consumption and mean body weight gain occurred in rats at all levels. All male and 4/5 female rats at 50,000 ppm died within 9 days.

Gross observations at necropsy revealed thymic atrophy, splenomegaly and dose-related darkening of the spleen, kidney and liver. Thymic atrophy was attributed to the low food consumption. Mice at ≥6000 ppm had dose-related decreased mean body weight gain. Three males at 25,000 ppm and two females at 50,000 ppm died within 12 days. At the high concentrations, mice had a hunched appearance and were lethargic, and spleens and kidneys were pigmented.

As reported in an abstract, Korosteleva and Chistyakova (1983) gave rats daily oral doses of 50 mg Direct Black 38 for 30 days and detected a benzidine antigen in the serum. The antigen was formed in the kidneys within 60 days.

5.7. SUMMARY

Direct Black 38 was demonstrated to be carcinogenic in mice and rats by the oral route. Mice exposed to 0.3% Direct Black 38 in their drinking water for 55-60 weeks had increased incidences of hepatomas and mammary carcinomas compared with controls (Asada et al., 1981). NCI (1978) observed a statistically significant increased liver tumor incidence for male and female rats given 1500 ppm of Direct Black 38 in the diet for 91 days. No liver tumors were observed at lower doses or in controls. Time to first tumor was 4 and 13 weeks in male and female rats, respectively, as compared with ~78 weeks in historic controls. No liver tumors were observed in mice; however, foci of cellular alteration of the liver were observed in three mice at 12,500 ppm. The duration of treatment may not have been long enough for mice to develop tumors.

IARC (1982a) reported several Japanese drinking water studies for Direct Black 38. Negative results were obtained in rats given 100 mg/ℓ for 60 weeks (Okajima et al., 1975) and 400 mg/ℓ for 14 months (Niitsu, 1973).

Two papillomas and three carcinomas of the urinary bladder, three carcinomas of the liver and two adenocarcinomas of the colon were obtained in 6/13 rats given 500 mg/ℓ for 60 weeks (Okajima et al., 1975). Negative results were obtained in a pulmonary adenoma bioassay in mice or when Direct Black 38 in wax pellets was implanted into the bladder of mice (Maronpot et al., 1983; Niitsu, 1973). Yoshida and Miyakawa (1972) and Yoshida et al. (1971) conducted a case control study of 200 males with bladder cancer and 148 male controls of the same age with urinary disorders. Seventeen of the bladder cancer cases and two of the controls worked in the silk-dyeing industry, [relative risk 6.8 (p=0.002)]. Seven of the 17 bladder cancer cases were silk painters who maintained oral contact with their work tools. Genin (1977) found bladder tumors in workers occupationally exposed to direct azo dyes and detected benzidine in the urine of exposed workers. IARC (1982a,b) concluded that exposures to benzidine-based dyes were strongly associated with the occurrence of bladder cancer in humans. Although the epidemiological data were inadequate to evaluate the possible carcinogenic effect to man of Direct Black 38 alone, when considered together with data demonstrating that 1) the formulation of Direct Black 38 contains varying amounts of residual benzidine, 2) that Direct Black has been shown to metabolize to benzidine, and 3) benzidine was present in the urine of occupationally-exposed workers, it provides an important correlation and evidence that occupational expusure to benzidine-based dyes represent a carcinogenic risk to man. In addition, there is a significant incidence of bladder cancers associated with exposure to benzidine.

Direct Black 38 was mutagenic in the Salmonella typhimurium reverse mutation assays (Prival et al., 1984; Joachim et al., 1985; Reid et al., 1984) and the presence of S-9 and FMN or bacterial reduction enhanced the mutagenic activity of the compound.

No data concerning the teratogenic effects of Direct Black 38 after oral or inhalation exposure was available. Wilson (1955) subcutaneously injected pregnant rats with 30 mg of Direct Black 38 and observed that 19/91 implantations were resorbed and no malformations occurred. Control data was not provided. Pertinent data regarding the reproductive effects of Direct Black 38 could not be located in the available literature as cited in the Appendix.

The subchronic toxicity of Direct Black 38 has been studied by NCI (1978) where rats and mice were exposed to concentrations of 190-3000 and 750-12,500 ppm, respectively. Among the effects observed in rats of both sexes were cirrhotic liver alterations including biliary hyperplasia at ≥750 ppm and nodular regeneration and portal fibrosis at 1500 ppm. Other toxic effects observed in rats were lymphoid depletion of the spleen and myeloid depletion of the bone marrow at ≥1500 ppm (both sexes), lymphoid depletion of the thymus in males at levels ≥1500 ppm and 3000 ppm in females, and subacute glomerulonephritis at 1500 ppm (both sexes). None of these lesions were found in controls. At 3000 ppm, all rats of both sexes died; six males at 1500 ppm died before the end of the study. Several toxic effects of Direct Black 38 were observed in mice. At concentrations ≥6000 ppm, mice of both sexes exhibited pigment deposits in the liver, hemosiderosis of the kidney and pigment deposits in the thyroid. At a concentration of 1500 ppm in mice of both sexes, hemosiderosis of the spleen was observed. Hepatocellular degeneration and biliary hyperplasia was observed in mice of both sexes at 3000 and 12,500 ppm, respectively.

6. AQUATIC TOXICITY

Little et al. (1974) reported that the 96-hour LC_{50} of Direct Black 38 to fathead minnows (Pimephales promelas) was >180 mg/ℓ. This greatly exceeds the concentrations used to produce color (0.4-1.5 mg/ℓ). No other information regarding aquatic toxicity of Direct Black 38 was found.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

NIOSH (1980) considered the benzidine-based dyes as potential human carcinogens and OSHA/NIOSH (1980) recommended that worker exposure be reduced to the lowest feasible level. In response to ITC recommendations, the U.S. EPA chose not to require additional testing of benzidine, o-tolui-dine and o-dianisidine-based dyes for health and environmental effects (U.S. EPA, 1981).

7.2. AQUATIC

Guidelines and standards for the protection of aquatic biota from the effects of Direct Black 38 or 3 could not be located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

Direct Black 38 was demonstrated to be carcinogenic in mice and rats by the oral route. Mice exposed to 0.3% Direct Black 38 in their drinking water for 55-60 weeks had increased incidences of hepatomas and mammary carcinomas (Asada et al., 1981). None of these tumors were observed in controls. NCI (1978) observed a statistically significant increased liver tumor incidence for male and female rats given 1500 ppm of Direct Black 38 in the diet for 91 days. No liver tumors were observed at lower doses or in controls. Time to first tumor was 4 and 13 weeks in male and female rats, respectively, as compared with ~78 weeks in historic controls. No liver tumors were observed in mice; however, foci of cellular alteration of the liver were observed in three mice at 12,500 ppm. The duration of treatment may not have been long enough for mice to develop tumors.

IARC (1982a) reported several Japanese drinking water studies for Direct Black 38. Negative results were obtained in rats given 100 mg/ℓ for 60 weeks (Okajima et al., 1975) and 400 mg/ℓ for 14 months (Niitsu, 1973). Two papillomas and three carcinomas of the urinary bladder, three carcinomas of the liver and two adenocarcinomas of the colon were obtained in 6/13 rats given 500 mg/ℓ for 60 weeks. Negative results were obtained in a pulmonary adenoma bioassay in mice and when Direct Black 38 in wax pellets was inplanted into the bladders of mice (Maronpot et al., 1983; Niitsu, 1973). Yoshida and Miyakawa (1973) and Yoshida et al. (1971) conducted a case control study of 200 males with bladder cancer and 148 male controls of the same age with urinary disorders. Seventeen of the bladder cancer cases and two of the controls worked in the silk-dyeing industry, where the relative risk was 6.8 (p=0.002). Seven of the 17 bladder cancer cases were silk

painters who maintained oral contact with their work tools. Genin (1977) found bladder tumors in workers occupationally exposed to direct azo dyes and detected benzidine in the urine of exposed workers. IARC (1982a,b) concluded that exposures to benzidine-based dyes were strongly associated with the occurrence of bladder cancer in humans. Although the epidemiological data were inadequate to evaluate the possible carcinogenic effect to man of Direct Black 38 alone, when considered together with data demonstrating that benzidine was present in the urine of occupationally exposed workers, it provided "sufficient evidence that occupational exposure to benzidine-based dyes represent a carcinogenic risk to man."

Direct Black 38 was mutagenic in the Salmonella typhimurium reverse mutation assays (Prival et al., 1984; Joachim et al., 1985; Reid et al., 1984), and the presence of S-9 and FMN or bacterial reduction enhanced the mutagenic activity of the compound.

No data concerning the teratogenic effects of Direct Black 38 after oral or inhalation exposure were available. Wilson (1955) subcutaneously injected pregnant rats with 30 mg of Direct Black 38 and observed that 19/91 implantations were resorbed and no malformations occurred. Control data was not provided. Pertinent data regarding the reproductive effects of Direct Black 38 could not be located in the available literature as cited in the Appendix.

The subchronic toxicity of Direct Black 38 has been studied by NCI (1978) where rats and mice were exposed to concentrations of 190-3000 and 750-12,500 ppm, respectively. Among the effects observed in rats of both sexes were cirrhotic liver alterations including biliary hyperplasia at $\geq$750 ppm and nodular regeneration and portal fibrosis at 1500 ppm. Other toxic effects observed in rats were lymphoid depletion of the spleen and myeloid

depletion of the bone marrow at 1500 ppm (both sexes), lymphoid depletion of the thymus in males at levels ≥1500 ppm and 3000 ppm in females, and subacute glomerulonephritis at 1500 ppm (both sexes). None of these lesions were found in controls. At 3000 ppm, all rats of both sexes died and six males at 1500 ppm died before the end of the study. Several toxic effects of Direct Black 38 were observed in mice. At concentrations ≥6000 ppm, mice of both sexes exhibited pigment deposits in the liver, hemosiderosis of the kidney and pigment deposits in the thyroid. At a concentration of 1500 ppm in mice of both sexes, hemosiderosis of the spleen was observed. Hepatocellular degeneration and biliary hyperplasia was observed in mice of both sexes at 3000 and 12,500 ppm, respectively.

Epidemiological studies demonstrated excessive bladder cancer cases in dyestuff workers (Yoshida and Miyakawa, 1973; Yoshida et al., 1971). This finding and the detection of benzidine and monoacetylbenzidine in the urine of workers occupationally exposed to benzidine-based dyes provide sufficient evidence that exposure to benzidine-based dyes is strongly associated with bladder cancer. Levels of benzidine, a known human carcinogen, in the urine of workers in a study by NIOSH (1980) were similar to or greater than levels in workers who were exposed to benzidine and who developed bladder cancer in a study by Zavon et al. (1973). The Zavon et al. (1973) study was used as the basis for the risk assessment for benzidine by U.S. EPA (1980c). Several authorities consider benzidine-based dyes to be potential human carcinogens (OSHA/NIOSH, 1980; NIOSH, 1980). IARC (1982a,b) concluded that while the evidence that Direct Black 38 is carcinogenic in humans is inadequate, there was sufficient evidence that benzidine-based dye exposure represents a carcinogenic risk for humans. IARC (1982a,b) also concluded that Direct Black 38 was carcinogenic in rats and mice by the oral route. Since Direct

Black 38 is considered by this assessment to be a human carcinogen as well as an animal carcinogen, it is appropriate therefore to derive a q_1^*. In the available epidemiological studies, workers were exposed to dye mixtures and estimated levels of exposure were not specified; therefore, they cannot be used in quantitative risk assessment. Two studies, Asada et al. (1981) and NCI (1978), provided data from which to derive a q_1^*. Asada et al. (1981) gave ICR mice 0.3% Direct Black 38 in the drinking water [570 mg/kg/day, calculated by multiplying the concentration of 3000 mg/ℓ by 0.0057 ℓ/day, the water consumption of a 0.03 kg mouse (U.S. EPA, 1986c) and dividing by the mouse body weight] for 55-60 weeks. Incidences of 46/59 and 20/59 for hepatomas and mammary tumors, respectively, and no tumors in a control group of 20 rats were observed. The liver tumor data were chosen to calculate a q_1^* for the Asada et al. (1981) study (Table 8-1). The unadjusted q_1^* of 3.44×10^{-3} $(mg/kg/day)^{-1}$ was calculated using the multistage model developed by Howe and Crump (1982). The human q_1^* of 2.7×10^{-1} $(mg/kg/day)^{-1}$ was calculated by multiplying the unadjusted q_1^* by the cube root of the ratio of the human body weight (70 kg) to the mouse body weight and by the cube of the ratio of the lifespan of the mouse (104 weeks) to the length of the experiment (57.5 weeks, the average of 55 and 60 weeks). NCI (1978) reported a liver tumor incidence of 9/9 and 5/10 for male and female rats given 1500 ppm of Direct Black 38 in the diet for 91 days. No liver tumors were observed in the controls. The q_1^* calculations are presented in Tables 8-2 and 8-3. The transformed doses were calculated by multiplying the dietary levels by 0.05 and by the cube root of the ratio of the rat body weight to the human body weight. This correction was made before calculating the q_1^* because the body weights of the different dose groups varied. The unadjusted q_1^*s were calculated without

TABLE 8-1

Cancer Data Sheet for Derivation of q_1^*

Compound: Direct Black 38

Reference: Asada et al., 1981

Species, Strain, Sex: mouse, ICR, male and female

Body weight: 0.03 kg (assumed)

Length of exposure (le) = 55-60 weeks

Length of experiment (Le) = 55-60 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: liver, hepatoma

Route, vehicle: oral, drinking water

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/20
3000	570	46/59

Unadjusted q_1^* from study = 3.439168×10^{-3} $(mg/kg/day)^{-1}$

Human q_1^* = 2.7×10^{-1} $(mg/kg/day)^{-1}$

TABLE 8-2

Cancer Data Sheet for Derivation of q_1^*

Compound: Direct Black 38

Reference: NCI, 1978

Species, Strain, Sex: rat, Fischer 344, male

Body weight: 0.22 kg controls, 190, 375 and 750 ppm groups; 0.16 kg 1500 ppm group; 0.10 kg 3000 ppm group

Length of exposure (le) = 91 days

Length of experiment (Le) = 93 days

Lifespan of animal (L) = 730 days

Tumor site and type: liver, hepatocellular carcinoma or neoplastic nodule

Route, vehicle: oral, diet

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/10
190	1.4	0/10
375	2.7	0/10
750	5.5	0/10
1500	9.9	9/9
3000	16.9	0/9

Unadjusted q_1^* from study = 1.5733×10^{-2} $(mg/kg/day)^{-1}$

Human q_1^* = 7.6 $(mg/kg/day)^{-1}$

NOTE: The q_1^* was calculated without the high-dose group (see text)

TABLE 8-3

Cancer Data Sheet for Derivation of q_1^*

Compound: Direct Black 38

Reference: NCI, 1978

Species, Strain, Sex: rats, Fischer 344, female

Body weight: 0.16 kg, control; 0.15 kg, 190 and 375 ppm groups; 0.14 kg, 750 ppm group; 0.11 kg, 1500 ppm group and 0.075 kg, 3000 ppm group

Length of exposure (le) = 91 days

Length of experiment (Le) = 93 days

Lifespan of animal (L) = 730 days

Tumor site and type: liver tumors

Route, vehicle: oral, dietary

Experimental Doses or Exposures (ppm)	Transformed Dose (mg/kg/day)	Incidence No. Responding/No. Tested or Examined
0	0	0/10
190	1.2	0/10
375	2.4	0/10
750	4.7	0/10
1500	8.7	5/10
3000	15.3	0/8

Unadjusted q_1^* from study = 1.80238×10^{-2} $(mg/kg/day)^{-1}$

Human q_1^* = 8.6 $(mg/kg/day)^{-1}$

NOTE: The q_1^* was calculated without the high-dose group (see text)

the high-dose groups because when the high-dose groups were included the data did not fit the model. The absence of tumors in the high-dose groups was attributed to the early mortality of the rats in these groups. The unadjusted q_1^*s were multiplied by the cube of the ratio of the lifespan to the length of the experiment to result in q_1^*s for humans of 8.7 and 7.6 $(mg/kg/day)^{-1}$ for the female and male rat data, respectively. The Asada et al. (1981) study might be preferable for quantitative risk assessment because the q_1^* estimates increased risk of cancer over a lifetime; therefore, a study that more closely approximates the lifespan of the species tested should be used. Further, the sample size used by Asada et al. (1981) was larger than that used by NCI (1978), and as stated by U.S. EPA (1980b), spuriously high q_1^*s may be obtained if studies with small sample sizes are used. Arguably, the human q_1^* derived from the NCI (1978) data is artificially high because the q_1^*s are multiplied by a very large number $(730/93)^3$ to correct for the short on-test period. Several reasons support the adequacy of the higher q_1^*, however. The NCI (1978) study is the better of the two studies because several doses were tested, the data were statistically analyzed, rats were tested with six concentrations of Direct Black 38 and the MTD was estimated by adequate pretesting. In Asada et al. (1981), only one concentration was used and adequate pretesting to estimate the MTD was not reported. In fact, these mice may not have been exposed to the MTD. As indicated by NCI (1978), the MTD may have been toward the higher end of the 750-12,500 ppm range tested. Therefore, 3000 ppm, which was sufficient to elicit a carcinogenic response, may not have been sufficient to adequately reflect the potency of the dye. Another reason for preferring the rat data to the mouse data is that rats may be the more sensitive species. High liver tumor incidences were

observed in rats in only 13 weeks, while mice only exhibited foci of cellular alteration at the highest dose, which possibly is an indication that a preneoplastic condition may develop into neoplasia with prolonged dosing (NCI, 1978). Data from Asada et al. (1981) indicate liver tumor development after 60 weeks of dosing to support this conclusion. If rats are more sensitive to the carcinogenic action of Direct Black 38, then it is obviously preferable to use the q_1^* for that more sensitive species. Implicit in this argument is the deduction that were a lower dose given to rats for a longer period of time the same response would occur. This conclusion is supported by a Japanese study (Okajima et al., 1975) reported by IARC (1982a), in which rats were given 100 and 500 mg/ℓ of Direct Black 38 in their drinking water for 60 weeks. No tumors were observed at the low dose or in the controls, but at 500 mg/ℓ 6/13 surviving rats had a total of 10 tumors. Thus, rats were tested at lower doses for longer periods of time and a carcinogenic response elicited. Therefore, the highest q_1^* of 8.6 $(mg/kg/day)^{-1}$ from the data for female rats in the NCI (1978) study is recommended for estimating the increased lifetime risk of cancer. The concentrations in water associated with risk levels of 10^{-5}, 10^{-6} and 10^{-7} are $4x10^{-5}$, $4x10^{-6}$ and $4x10^{-7}$ mg/ℓ, assuming a 70 kg human consumes 2 ℓ water/day.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Chronic oral or inhalation studies of Direct Black 38 were not available; however, NCI (1978) adequately described the subchronic oral toxicity of Direct Black 38 (see Section 5.5.). The data provided was sufficient to determine human MEDs, CSs and RQs. Relevant information such as animal exposure levels, transformed doses and toxic effects are summarized in Table 9-1.

As seen from Table 9-1, some of the effects of subchronic oral exposure to Direct Black 38 include cirrhotic liver alterations, lymphoid depletion of the spleen and myeloid depletion of the bone marrow, increased mortality, hemosiderosis of the spleen, hepatocellular degeneration and glomerulonephritis. Several effects reported in mice (pigment deposits in the liver and thyroid hemosiderosis of the kidney, biliary hyperplasia) were also not considered in Table 9-1 because they occurred at the two highest levels (6000 and 12,500 ppm). Since exposures were subchronic, the dose was divided by a factor of 10 to approximate chronic exposure. As seen from Table 9-1, cirrhotic liver alterations were observed at the lowest equivalent chronic human dose (0.55 mg/kg/day). These effects were given an RV_e of 7 (Table 9-2). Increased mortality was observed at a higher equivalent chronic human dose (1.0 mg/kg/day). Since the severity (RV_e=10) of this effect was higher than the RV_e for cirrhotic liver changes, it was also included in Table 9-2. Upon calculation of the equivalent chronic human dose several effects could be eliminated from consideration in calculating CSs (see Table 9-2). Lymphoid depletion of the spleen and myeloid depletion of the bone marrow and glomerulonephritis would have been given an RV_e of <7, but they occurred at higher equivalent chronic human doses. Therefore,

TABLE 9-1

Oral Toxicity Summary for Direct Black 38 Administered in the Diet[a,b]

Species/Strain	Sex	No. at Start	Average Weight[c] (kg)	Exposure	Transformed Animal Dose[d] (mg/kg/day)	Equivalent Chronic Human Dose[e] (mg/kg/day)	Response
Rat/Fischer 344	M	10	0.22	750 ppm in diet for ~91 days	3.75	0.55	Cirrhotic liver alterations
Rat/Fischer 344	F	10	0.15	750 ppm in diet for ~91 days	3.75	0.48	Cirrhotic liver alterations
Rat/Fischer 344	M	10	0.16	1500 ppm in diet for ~91 days	7.5	1.0	Increased mortality; lymphoid depletion of the spleen and myeloid depletion of the bone marrow; cirrhotic liver alteration; glomerulonephritis
Rat/Fischer 344	F	10	0.11	1500 ppm in diet for ~91 days	7.5	0.87	Lymphoid depletion of the spleen and myeloid depletion of the bone marrow; cirrhotic liver alteration; glomerulonephritis
Mouse/B6C3F1	M	10	0.026	1500 ppm in diet for ~91 days	19.5	1.4	Hemosiderosis of the spleen
Mouse/B6C3F1	F	10	0.020	1500 ppm in diet for ~91 days	19.5	1.3	Hemosiderosis of the spleen
Mouse/B6C3F1	M	10	0.026	3000 ppm in diet for ~91 days	39.0	2.8	Hepatocellular degeneration
Mouse/B6C3F1	F	10	0.020	3000 ppm in diet for ~91 days	39.0	2.6	Hepatocellular degeneration

[a]Source: NCI, 1978

[b]Purity = benzidine-free

[c]Estimated from body weight data provided by NCI, 1978

[d]Calculated by multiplying dietary level by a food factor of 0.05 for rats, 0.13 for mice (U.S. EPA, 1985) and dividing by a factor of 10 for a subchronic study.

[e]Equivalent chronic human doses were calculated by multiplying the transformed animal dose by the cube root of the ratio of the average animal body weight to the reference human body weight (70 kg).

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TABLE 9-2

Oral Composite Scores for Toxicity of Direct Black 38
Using Fischer 344 Rats[a]

Animal Dose (mg/kg/day)	Chronic Human MED[b] (mg/day)	RV_d	Effect	RV_e	CS	RQ
3.75	38.5	3.1	cirrhotic liver alterations	7	22	100
7.5	70.0	2.7	increased mortality	10	27	100

[a]Source: NCI, 1978

[b]The dose was divided by an uncertainty factor of 10 to approximate chronic exposure

a CS was not calculated for these effects. Hemosiderosis of the spleen and hepatocellular degeneration in mice were also omitted from Table 9-2 because these effects occurred at higher equivalent chronic human doses and were of similar or lesser severity than the effects given in Table 9-2. As can be seen, the effect of cirrhotic liver alterations was given an RV_e of 7 and an RV_d of 3.1, yielding a CS of 22. A CS (27) was obtained for the effect of increased mortality with an RV_d of 2.7 and an RV_e of 10. These CSs correspond to RQs of 100, but the higher CS was obtained for increased mortality (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Studies regarding the carcinogenicity of Direct Black 38 were discussed in Section 5.1. Epidemiological studies have demonstrated excessive bladder cancer cases in dyestuff workers (Yoshida and Miyakawa, 1973; Yoshida et al., 1971). This finding, considered with evidence that benzidine and monoacetylbenzidine were detected in the urine of workers occupationally exposed to benzidine-based dyes provides sufficient evidence that exposure to benzidine-based dyes is strongly associated with bladder cancer. Levels of benzidine, a known human carcinogen, in the urine of workers in a survey by NIOSH (1980) were similar to or greater than levels in workers who were exposed to benzidine and who developed bladder cancer in the study by Zavon et al. (1973). The Zavon et al. (1973) study was used as the basis for the risk assessment for benzidine by U.S. EPA (1980c). Several authorities consider benzidine-based dyes to be potential human carcinogens (OSHA/NIOSH, 1980; NIOSH, 1980). IARC (1982a,b) concluded that while the evidence that Direct Black 38 is carcinogen in humans is inadequate, there was sufficient evidence that benzidine-based dye exposure represented a carcinogen risk for humans. IARC (1982a,b) also concluded that Direct Black 38 was carcinogenic by the oral route to rats and mice (see Section 5.1.). Therefore, it is

TABLE 9-3

Direct Black 38

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	dietary
Dose*:	70 mg/day
Effect:	increased mortality
Reference:	NCI, 1978
RV_d:	2.7
RV_e:	10
Composite Score:	27
RQ:	100

*Equivalent human dose

appropriate to derive a potency factor. As discussed in Chapter 8, a q_1^* of 8.6 $(mg/kg/day)^{-1}$ was derived based on the incidence of liver tumors in female rats in the NCI (1978) study. A potency factor (F) can be derived from the same data (Table 9-4). The transformed doses were calculated by multiplying the dietary levels by 0.05 and by the cube root of the ratio of the rat body weight to the human body weight. The body weight correction was made before calculating the $1/ED_{10}$ because the weights in the different groups varied. By using the multistage model (Howe and Crump, 1982), an unadjusted $1/ED_{10}$ of 1.78×10^{-1} $(mg/kg/day)^{-1}$ was obtained. Correcting by the cube of the ratio of the length of rat lifespan to the duration of the study to adjust for the time on test results in the F factor of 86 $(mg/kg/day)^{-1}$. Because F is >10, Direct Black 38 is placed in Potency Group 2.

A consideration of the metabolism and related factors is important in that the formulation of Direct Black 38 contains apparently varying amounts of residual benzidine as a contaminant and that animals including monkeys have been shown to metabolize Direct Black 38 to benzidine and monoacetal-benzidine to some degree. The presence of benzidine in the urine of workers exposed to benzidine-based dyes together with the significant incidence of bladder cancer in humans exposed to benzidine is an important correlation.

Although animal evidence is limited, benzidine is an EPA Group A compound, a known human carcinogen (U.S. EPA, 1986b). Therefore, the animal evidence together with the knowledge that Direct Black is metabolized to benzidine and that the formulation of Direct Black 38 contains varying amounts of residual benzidine, elevates the weight of evidence for benzidine-based dyes to a Group A compound. Direct Black 38 has been classified by IARC as Group 2A. A potency Group 2 and U.S. EPA Group A chemical has a HIGH hazard ranking under CERCLA.

TABLE 9-4

Derivation of Potency Factor(F) for Direct Black 38

Reference:	NCI, 1978
Exposure route:	oral
Species:	rats
Strain:	Fischer 344
Sex:	female
Vehicle or physical state:	dietary
Body weight:	0.16 kg, controls; 0.15 kg, 190 and 335 ppm groups; 0.14 kg, 750 ppm group; 0.11 kg, 1500 ppm group and 0.075 kg, 3000 ppm group
Duration of treatment:	91 days
Duration of study:	93 days
Lifespan of animal:	730 days
Target organ:	liver
Tumor type:	neoplastic nodules
Experimental doses/exposures:	0, 190, 375, 750, 1500, 3000 ppm
Transformed doses (mg/kg/day):	0, 1.2, 2.4, 4.7, 8.7, 15.3
Tumor incidence:	0/10, 0/10, 0/10, 0/10, 5/10, 0/8
Unadjusted $1/ED_{10}$:	1.78×10^{-1} $(mg/kg/day)^{-1}$
Adjusted $1/ED_{10}$ (F factor)*:	86 $(mg/kg/day)^{-1}$

*The F factor was calculated without the high-dose group because when the high-dose group was included, the data did not fit the model.

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Yoshida, O., T. Hurada, M. Miyagawa and T. Kato. 1971. Bladder cancer in workers of the dyeing industry -- Epidemiological survey focusing on Kyoto refecture. Igaka no Ayumi. 79: 421-422. (Cited in IARC, 1982a)

Yoshida, O., M. Miyakawa, Y. Okada, et al. 1981. The disintegration of a benzidine dye, direct deep black ex by escherichia coli and bacteria. In: Decomposition of Toxic and Nontoxic Organic Compounds in Soil, M.R. Overcash, Ed. Ann Arbor Sci. Publ., Ann Arbor, MI. p. 227-231.

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

GLOBAL
TSCATS
CASR online (U.S. EPA Chemical Activities Status Report)
CAS online STN International
TOXLINE
TOXBACK 76
TOXBACK 65
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP
NTIS
Federal Register

These searches were conducted in May, 1986. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1986. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 1985-1986. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1985-1986. Cincinnati, OH. 114 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

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Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., Littleton, MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1986. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

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Sax, N.I. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nostrand Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Menlo Park, CA.

U.S. EPA. 1985. Status Report on Rebuttable Presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1985. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (U.S. International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publ. 1422, Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book. Volume 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under Contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California, State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. Prepared for the U.S. EPA, Washington, DC. PB-269605.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. Office of Pesticide Programs, U.S. EPA, Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.

In addition, the following documents were consulted during preparation of this HEEP:

U.S. EPA. 1977. Notice of Rebuttable Presumption Against Registration and Continued Registration of Pesticide Products Containing Lindane. OPP. Federal Register. 42(33): 9816-9947.

U.S. EPA. 1980. Preliminary Notice of Determination Concluding the Rebuttable Presumption Against Registration of Pesticide Products Containing Lindane; availability of Position Document 2/3. OPP. Federal Register. 45(130): 45362-45369.

U.S. EPA. n.d. Lindane Position Document 4. OPP, U.S. EPA, Washington, DC. Master Copy.